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Identification of protease exosite-interacting peptides that enhance substrate cleavage kinetics.

Journal: Biol Chem

Publication Year: 2012

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PubMed link: 22944693

Public Summary:

Scientific Abstract:
Many peptidases are thought to require non-active site interaction surfaces, or exosites, to recognize and cleave physiological substrates with high specificity and catalytic efficiency. However, the existence and function of protease exosites remain obscure owing to a lack of effective methods to identify and characterize exosite-interacting substrates. To address this need, we modified the cellular libraries of peptide substrates (CLiPS) methodology to enable the discovery of exosite-interacting peptide ligands. Invariant cleavage motifs recognized by the active sites of thrombin and caspase-7 were displayed on the outer surface of bacteria adjacent to a candidate exosite-interacting peptide. Exosite peptide libraries were then screened for ligands that accelerate cleavage of the active site recognition motif using two-color flow cytometry. Exosite CLiPS (eCLiPS) identified exosite-binding peptides for thrombin that were highly similar to a critical exosite interaction motif in the thrombin substrate, protease-activated receptor 1. Protease activity probes incorporating exosite-binding peptides were cleaved ten-fold faster than substrates without exosite ligands, increasing their sensitivity to thrombin activity in vitro. For comparison, screening with caspase-7 yielded peptides that modestly enhanced (two-fold) substrate cleavage rates. The eCLiPS method provides a new tool to profile the ligand specificity of protease exosites and to develop improved substrates.

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